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A comparative study of inoculation with two growth-promoting and siderophore-producing bacteria (*Enterobacter cloacae* & *Bacillus cereus*) on iron availability in pistachio seedlings *(Pistacia Vera* L.)

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Introduction

Iron deficiency is one of the most important problems that affect the growth of plants. Trivalent iron (Fe^{3+}) is not absorbable form for plants and divalent iron in the form (Fe^{2+}) is the main form of iron absorbable by plants, that's why many plants suffer from iron deficiency and these plants must be fertilized to eliminate the deficiency. Siderophores and phytosiderophores are special Fe^{3+} chelators with low molecular weight (less than 1500 Da) that form complexes with free iron and bring it into the cell through membrane receptors. Some microorganisms have evolved mechanisms that cause iron absorption, these microorganisms increase iron absorption by producing siderophores that have a strong tendency to bind to iron. Siderophore-producing bacteria include both gram-positive and gram-negative bacteria. The siderophore-iron complex is formed in bacteria and a reduction process occurs in the bacterial membrane and Fe^{2+} is converted to Fe^{3+} and becomes available to the plant (Sah & Singh, 2015).

Pistachio is one of the most important agricultural products and about 80 percent of its cultivated and production area is in Kerman province. The research conducted on the nutritional status of pistachio trees in pistachio farming areas of Kerman province has shown that pistachio orchards with low yield generally have the order of nutrient requirements Mn>Zn>K>Fe>N>Cu>P>Ca >B>Mg, in this sequence, Fe is the fourth priority in terms of deficiency in gardens with low yield (Hosseini Fard et al. 2016).

Considering the importance of iron element in the pistachio farming areas of Iran and the limitation in the absorption conditions of this element from the calcareous soils of these areas, an important option to increase the quality and performance of pistachio plant can be the use of growth stimulating bacteria that has the ability to produce siderophore. The aim of this research project was to investigate the effects of two strains (*Enterobacter cloacae* and *Bacillus cereus*) of siderophore-producing root growth stimulating bacteria in increasing the availability of iron in pistachio seedlings.

Material and Methods

This research was performed in a completely randomized factorial design. Ferric iron (Fe^{+3}) and ferrous iron (Fe^{+2}) were treated on seedlings. The sterilized pistachio seeds were soaked in water for twenty-four hours and then they were placed in a wet cotton cloth and the cloth was changed once a day. After germination, they were placed in bacterial suspension for 30 minutes and planted one in each pot containing sieved, acid-washed, and autoclaved air sand. After germination of the seeds, 20 cc of the bacterial suspension was given to each pot once a week (this continued for a month). The bacteria were grown in Nutrient Broth Agar culture medium in a Petri dish in an incubator with a temperature of 28-30 degrees Celsius for 24 hours, and after growth, the solid culture medium was kept in the refrigerator. To transfer the bacteria to the soil, first, the grown bacteria were transferred from the solid culture medium to the liquid medium and placed in a shaker-incubator for 24 hours until they grew and the bacterial suspension was prepared. To ensure the production of siderophore in the bacteria used, the siderophore test was performed.

After optimizing iron concentrations, the 20 μM was selected. The iron solution was given once a week to the seedlings that had grown for a month. Treatments were done for 4 weeks. For iron treatment, 30 cc of 20 micromolar ferric and ferrous iron treatment were given to the pots. Seedlings without iron (zero iron) were considered as controls. After two months of planting, physiological, biochemical and growth parameters were investigated.

Statistical analyzes of the data obtained from various quantitative measurements in this study were done using SPSS software and the analysis of variance method, and the differences between the means were compared using the LSD test at the 95% confidence level. The relevant figures were drawn with Excel 2007 software.

Results & Discussion

By using growth-promoting bacteria in ferric iron-treated plants, some growth parameters such as fresh weight, photosynthetic pigments, phenolic compounds, soluble sugars, lipid peroxidation and proline were improved. Treatment of plants with growth-promoting bacteria also increased the amount of protein in plants treated with ferric iron. Growth-promoting bacteria also increased iron uptake in the shoots of ferric iron-treated seedlings. Results of the siderophore amount showed that the bacteria were capable of producing siderophore, and probably their role in compensating for the decrease in growth caused by iron deficiency is for this reason. The increase in the amount of total iron in the leaves observed in this study can be due to the positive performance of bacteria in providing absorbable iron in pistachio plants. Perhaps one of the reasons for the increase in the chlorophyll content of plants after the application of growth-promoting bacteria in conditions of iron deficiency is due to the effective role of bacteria in providing iron along with protecting the membrane and chloroplast structure by eliminating reactive oxygen species. It has been reported that growth-promoting bacteria compensate for iron deficiency by converting ferric iron to ferrous iron by producing siderophore and provide absorbable iron to the plant and prevent membrane damage caused by iron deficiency. By reducing the amount of malonaldehyde, they play a protective role for the membrane (Shukla et al., 2012).

In this research, it was observed that in the treatment of non-absorbable iron, the use of PGPR bacteria increased the amount of carbohydrates compared to plants without bacteria treatment. This effect of bacteria in increasing the amount of carbohydrates has also been reported in previous studies and they have stated that this effect can be due to the provision of iron available to the plant, increasing the amount of chlorophyll and subsequently increasing photosynthesis and synthesis of sugars, reducing oxidative stress and Protection of chloroplast membrane and cell membrane. The increase in protein and proline content after the use of bacteria in iron deficiency conditions is probably due to the effective role of bacteria in improving the antioxidant capacity and preventing oxidative damage to proteins and their destruction. It seems that in plants treated with ferric iron, growth-promoting bacteria with the production of siderophore led to the reduction of iron and increase the available iron in the plant and reduce iron deficiency stress.

Conclusion

In this study, an increase in the amount of plant phenols was observed in ferric iron treatment. The secreted phenols can increase protons and convert ferric iron to ferric iron, which can be absorbed by the plant. Generally, it seems that bacteria provide absorbable ferrous iron to the plant by producing siderophore and creating a ferric iron-siderophore complex and reducing ferric iron to ferrous iron. Therefore, in iron-deficient plants, PGPR bacteria can be used to biologically restore iron and provide it to the plant and compensate for the reduction in iron nutrition.

Key words: *Ferric iron, Plant growth promoting bacteria, Lipid peroxidation, iron deficiency*

Declaration of conflict of interest

The authors declare that they have no conflicts of interest.